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Electrophysiological Assessments of Time- Course Effects on Diabetic peripheral Neuropathy and Their Correlation to the Current Hypotheses

دراسة كهرو – فسلجية لأعتلال الأعصاب المحيطة لمرضى داء السكري في ضوء نوع وأمد الأصابة

I. W. Muflih*, A.M. Abdul-Kareem & G.A. Al-Sharify*****

**Faculty of Pharmacy, An-Najah National University, Nablus, Palestine,*

***Sadam College of Medicin, Baghdad, ***Al-Yarmouk Hospital, Baghdad.*

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Abstract

Diabetes mellitus patients (number = 228) of insulin dependent (IDDM, two groups) and non- insulin dependent (NIDDM, two groups) were enrolled in the present study. Each of the two groups of the patients were comprising a group of less than ten years and another group of more than ten years duration of the disease.

Seven peripheral nerves were tested electrophysiologically for their latency, velocity and amplitude.

The obtaiened results showed significant differences between the two types of the disease durations. Measurments in terms of increased latencies with reduction in the sensory and motor conductive velocities, along with reduction in amplitudes were found more evident in diabetic patients with more than ten years duration in comparison to patients of less than ten years duration of the disease.

The obtained results of the present work suggests a recommendation for the mixed metabolic- vascular pathogenesis hypothesis that concern the diabetic neuropathy.

Key words: Time – course effects on diabetic neuropathy.

ملخص

أشتملت الدراسة على أربع مجموعات من مرضى السكري، مجموعتين منها للمرضى منذ عشرة سنوات تقريباً ومجموعتين أخريين للمرضى منذ أكثر من عشر سنوات وتم مقارنة نتائج التجارب مع مجموعة خامسة من الأصحاء.

أجريت الفحوصات والتجارب على سبعة أعصاب طرفية موزعة في الأطراف العليا والسفلى وذلك بهدف معرفة التأثيرات الأمراضية التي تكتنف اضعاف الأداء الوظيفي للأعصاب عند مرضى السكري وتم ذلك باستدلال القياسات الكهرو - فسيولوجية .

لقد أظهرت النتائج فروقات ذات مدلولات واضحة للتأثير الزمني للمرض على أعصاب المرضى لعشر سنوات والمرضى لأكثر من عشر سنوات، كذلك اتضح ازدياد زمن الكمون الحسي والحركي وانخفضت سرعة نقل الايعازات العصبية كما انخفض المدى الكامل للأستجابة العصبية في اعصاب المرضى المصابين منذ اكثر من عشر سنوات عنه في أعصاب المرضى المصابين منذ حوالي العشر سنوات فقط.

أوضحت مدلولات الدراسة أن فرضية التأثير الأمراضي الأيضي الوعائي المشترك هي الأرجح في عملية خفض الأداء الوظيفي للأعصاب الطرفية لدى مرضى السكري .

Aims of the study

It has been reported [1] that the precise pathogenesis of diabetic peripheral neuropathy remains poorly understood. In recent years many mechanisms have been proposed [2] for the etiology of diabetic neuropathy.

The present study is a trail to assist the effectiveness of time- course on the functional activity of seven peripheral nerves in the upper and lower limbs.

Some additional knoweledge, by use the electrophysiological technique, may arise for the progress of the current hypotheses in diabetic neuropathy.

Introduction

The current hypotheses of etiology of diabetic neuropathy are the followings:

1. **Metabolic hypothesis:** Also known as the polyol hypothesis. The brief outline of this hypothesis is that the unused glucose in diabetes is shunted into the polyol pathway and converted to sorbitol and frunctose by the enzymes aldose reductase and sorbitol dehydrogenase [3,4]. Accumulation of sorbitol and fructose in the

peripheral nerves, as in other tissues, leads to sturctural changes and breakdown of the nerve. [5-8].

2. **The vascular "ischemic- hypoxic" hypothesis:** this hypothesis stresses on the very early development of reduced endoneural blood flow, increased endoneural vascular resistance, reduced endoneural oxygen tension and the importance of these factors in the pathogenesis of diabetic neuropathy [9-11). Increased blood viscosity and decreased erythrocyte deformability in diabetes are contributing still further to the nerve ischemic hypoxic hypothesis [9]. Excessive formation of advanced glycosylation end product (AGE) in the cytoplasm of endothelial cells, pericytes, axoplasm and shwann cells were corellated with reduction in myelinated fiber density and formed deposition may play a role in the development of diabetic neuropathy [12].

3. **Growth factors hypothesis**

   A. Nerve growth factor (NGF)

   B. This hypothesis implicates the reduction of endogenous concentration of growth factors, particularly of nerve growth factor, which shares several molecular, structural and physiologic properties with insulin [13-15]. Neuronal growth factors promote the survival maintenance and regeneration of nuronal structure, therefore the abnormal expression of these factors in diabetes may lead to the impaired maintenance of normal nerve morphology and function [16].

   C. b. Insulin – like growth factor (IGF): Insulin- like growth factors I & II have also been implicated in the pathogenesis of diabetic peripheral neuropathy. IDDM patients who have decreased levels of IGF-I correlates with marked impairment of peripheral nerve regeneration [17]. Insulin is an important determinant of the concentration of IGF-I level in the tissues and plays a role in the regulation of IGF receptors [18].

Control of blood glucose level

The role of normalizing blood glucose concentration for controlling the diabetic complication is not fully elucidated. However there are increasing evidence that the probability of developing microvascular complications, including neuropathy is reduced by good glycemic control [19]. This is difficult to prove because of the difficulty in normalizing blood sugar in the diabetic patients all the time and the lack of well-defined end points [20]. The DCCT [21] study suggests that normalization of blood glucose levels have a major effect in preventing microvascular complications, including neuropathy.

Methods and Techniques

A control group of healthy individuals (30 males and 30 females) of comparable age group to the patients and free of impaired glucose tolerance test ensured by OGTT [22] were enrolled in the presnet study. They were of normal weight – height ratio, on usual diet with no drug taken at the time of examination. Renal and liver function tests were normal.

Four groups of the patients were enrolled to study the effects of duration of diabetes mellitus on nerve function (Table1) the average age of the enrolled patients were (37 ± 15) years who were attending Al- Yarmouk Teaching Hospital.

Table 1: Diabetic patients according to duration of the disease.

Group		Criteria	η
1	A	IDDM patients < 10 years duration	50
	B	IDDM patients > 10 years duration	50
2	A	NIDDM patients < 10 years duration	78
	B	NIDDM patients > 10 years duration	50

Examination of tested nerve functions were carried out one time for the control subjects and one time for the diabetic patients at the first visit. On

each visit the ulnar, median sural, common peroneal and posterior tibial nerves were tested for latency, conduction velocity and amplitude.

The Neuromatic 2000c apparatus, used in the present study, is a fully equipped two-channel neuromyograph for clinical EMG, NCS and evoked response. It is a microcomputer controlled instrument, comprising an active electrode box with patient-isolated inputs, EMG-amplifier, averages, monitor loudspeaker, chart recorder and stimulator for NCS. The monitor has a wide range of sweep setting and digital display of latency and duration.

Action potentials form nerves and muscles which evoked by applying pulses from the stimulator were recorded by the electrode tips and were fed to the EMG amplifier which is provided by calibration facilities.

Types of the used Electrodes

1. Stimulating Electrode: The 13L 36 surface stimulating electrode is a bipolar surface electrode for stimulating both the motor and sensory nerves through the skin.
2. Recording Electrode: The 13L 37 surface recording electrode is a bipolar surface electrode for the recording of sensory and motor nerve conduction through the skin.
3. Concentric Needle Electrode: The 13L 49 and 13L 58 concentric needle electrode consists of an isolated platinum wire located inside a stainless steel cannula and embeded in Araldite. The muscle action potentials are picked up by the tip of the platinum wire and the cannula as the indifferent electrode.
4. Grounding Electrode: The 13L 80 grounding plate electrode is made of stainless steel.

The 13L 38 Fixation starp is used to mount the stimulating electrode 13L 36, recording electrode 13L 37. and grounding plate electrode.

Recordings and Measurements

1. Motor nerve conduction recordings (MNCR):
   The motor latency was measured to the nearest 0.1 msec from the onset of the stimulus artifact to the initial of the deflection from the baseline of the muscle action potential. The conduction time between the two stimulating points and the difference between the proximal and distal latencies were recorded directly on the screen. The distance between the center of the stimulating cathodes, as measured on the skin following the course of the nerve was taken as the nerve conduction distance. The motor conduction velocity (MCV) was calculated by dividing the conduction distance (in meters) by the conduction time (in seconds).

2. The sensory nerve conduction recordings (SNCR):
   The sensory latency was measured from the onset of the stimulus artifact to the initial positive peak of the sensory action potentail (SAP) the nerve conduction distance was measured on the skin between the center of the stimulating cathode. The center of sensory conduction velocity (SCV) was calculated directly by dividing the nerve conduction distance (in meters) by the latency value (in second). The amplitude of the nerve action potential was measured from peak to peak (positive to negative deflection from the baseline).

The skin was carfully prepared at both the stimulating and the recording sites. It was disinfected by 70% alcohol and gently rubbed to decrease its resistance to the applied current. Maximum response was usually obtained by a current ranged 40-60 and 15- 25 m Amp when testing motor and sensory fibers respectively. All the nerves recordings were carried out on the limbs of the right side of the patients. During examination of the median and ulnar nerves the subject lies supine with the arms extended 180° at the elbow. The knee bent 120° when the peroneal and posterior tibial nerves were examined. When examined sural nerve, the subject lies prone with a support under the ankle with the knee at an angle of 140 [23].

The stimulation was square wave pulses of negative polarity with a duration of 0.2 msec at a rate of one per second. The sural nerve was stimulatd anti- dromically just lateral to the midline of the calf, 17 cms proximal to the recording electrode, which was placed just behind the lateral malleolus [24]. The ground electrode is placed between the cathode and the active pickup electrode.

Stimulation of the common peroneal nerve was done at two points, the first at the ankle between the two malleoli and the second site is just at the lateral side of the popliteal fossa at the fibular head. Stimulation was done by surface electrode 13L 36 and the evoked muscle action potential was recorded by the recording surface 13L37 from the extensor digitorum brives muscle. The conduction velocity was obtained by dividing the average distance between the two points of stimulation by the conduction time between these two points.

The posterior tibial nerve stimulaion was carried out behind medial malleolus as a distal point, and at the central position of the popliteal fossa as a proximal point. The stimulation was performed using the surface electrode 13L 36 and the evoked muscle action potential was recorded from the abductor hallucis muscle. The conduction velocity was obtained by dividing the distance between the two points of stimulation by the conduction time.

The ulnar sensory nerve was stimulated by using the surface electrode 13L 36 which was applied 14 cm proximal to the interphalangeal joint of the little finger and the grounding electrode was placed at the wrist between the stimulation and the recording electordes.

The median sensory nerve was stimulated by using the surface electrode 13L 36 which was placed 14 cm proximal to the interphalangeal joint of the index finger. The recording electrode was placed at the proximal interphalangeal joint of the index finger. The grounding electrode was placed at the wrist between the stimulating and recording electrodes.

The median motor nerve was stimulated at two points, the first is at the wrist and the second one is near the elbow, on the medial side just before the median nerve enters between the two heads of pronator teres muscle.

The evoked muscle action potential was recorded form the abductor pollicis brevis muscle. The motor conduction velocity was obtained by dividing the distance betwenn the two points of stimulation by the conduction time.

Stimulation of the ulnar motor nerve using the surface electrode was done at two points. The first is at the wrist about 8 cm porximal to the recording electorde which was placed on the abductor digiti minimi muscle. The second point was placed at two cm distal the medial epicondyle. The distal motor latency was recorded at the wrist and the conduction velocity was obtained by dividing the distance between the two stimulating points by the conduction time.

All the recordings were performed in an air- conditioned room with a temperture of $23 \pm 2^{\circ}$C and the skin temperature of the examined subjects were $34 \pm 1.5^{\circ}$C, for the lower limb and $35 \pm 1.4^{\circ}$C for the upper limb.

Statistics

Statistical analysis of the obtained recordings was carried out by feeding the data into IBM computer. The arithmetic mean, standard deviation (SD) and correlation coefficient (γ) were calculated by using statistical methods [25]. Student’s paired and unpaired t- test were used to analyze the difference between the subgroups concerning the P- values, any value greater than 0.05 was regarded as insignificant.

Results

1. The presentation of tested parameters, latency, velocity and amplitude follow always this sequences.
2. The examined parameters in the present paper are mentioned in the text without their units for simplicity. The units for latency in millisecond, for velocity meter per second and for amplitude is millivolt.
3. Values are expressed as mean + SD in the tablets but in the text values are expressed by their mean only.

4. The data of tested parameters of the patients visits were compared to that of the control group.

Latency, volocity and amplitude of the tested nerves of the healthy control show no significant differences between males and females, so they were regareded as one group. (Table 2).

In order to clarify the effects of DM duration on the nerve function, a hundred insulin dependent diabetic patients (IDDM) were tested (Table3). They were divided into A and B groups. The A are less than 10 years, and the B are more than 10 years durations. Comparison between the two groups for the latency, velocity and amplitude of the nerves were carried out. The same procedure was used to evaluate the effect of duration of DM (Table 4) on 108 NIDDM patients (group A, η = 50).

A: Insulin dependent diabetic patients (Table 3)

1. Sural nerve

Recordings from this nerve in patients with less than 10 years duration (group A) were 4.8, 38.38 and 5.3, while the recordings from group B(> 10 years duration) were 4.56, 35.4 and 3.8 , the P values are < 0.01, < 0.05 and <0.05 respectively for latency, velocity and amplitude.

2. Common Peroneal nerve

Recordings in group A were 4.86, 39.7 and 3.69 while in group B were 5.29, 36.12 and 3.0 with P value <0.05 . 0.05 and 0.025 respectively for the three parameters.

3. Posterior Tibial nerve

Recordings of this nerve in group A were 4.64, 38.9 and 3.58 while in group B were 5.25, 36.41 and 3.2, showing P value of < 0.05 for latnecy and velocity while the amplitude showed no P value.

4. **Median nerve – Sensory**

   Recordings from this nerve in group A were 3.82 and 40.1 and 4.91 while in group B were 4.02, 39.2 and 4.42 with P value insignificant for latency and P value < 0.025 for velocity and amplitude.

5. **Median nerve- Motor**

   Recordings in group A were 3.44, 42.93 and 4.8 while in group B were 4.67, 40.2 and 4.4 with P value < 0.025 for latency and amplitude while P value for velocity was < 0.05.

6. **Ulnar nerve – Sensory**

   Recordings of this nerve in group A were 2.99, 43.13 and 5.23 while in group B were 3.21, 40.55 and 5.6 showing P value < 0.01, 0.05 and 0.025 for the three parameters respectively.

7. **Ulnar nerve – Sensory**

   Recordings of this nerve in group A were 2.98, 45.7 and 5.27 while in group B were 3.7, 43.9 and 5.0 showing P value < 0.05, 0.05 and > 0.05 for the three parameters in the respective order.

B: Non- Insulin dependent diabetic patients (Table 4)

1. **Sural nerve**

   Recording of this nerve for group A were 4.7, 38.75 and 4.02, while the recordings of group B were 5.1, 35.32 and 3.68, the P value was < 0.05, 0.005 and 0.05 for latency, velocity and amplitude in the respective order.

2. **Common Perneal nerve**

   Recordings in group A were 4.9, 42.2 and 3.5, while in group B were 5.26, 36.5 and 3.2 with P value < 0.05,

0.001 and > 0.05 for the three parameters in the respective order.

3. Posterior Tibial nerve

Recordings of this nerve, group A were 5.3, 41.78 and 3.62 while in group B were 5.8, 36.3 and 3.2, showing P value of < 0.025 for latency and < 0.05 for velocity while P value for amplitude was insignificant.

4. Median nerve- Sensory

Recordings of this nerve in group A were 3.75 and 41.5 and 5.1 while in group B were 4.25, 37.2 and 4.5 with P value < 0.05, 0.005 and 0.05 for the three parameters respectively.

5. Median nerve- Motor

Recordings in group A were 4.27, 47.2 and 4.32 while in group B were 4.86, 38.32 and 3.0 with P value < 0.05, 0.005 and < 0.05 for the three parameters respectively.

6. Ulnar nerve- Sensory

Recordings of this nerve in group A were 3.2, 42.5 and 6.0 while in group B were 3.6, 39.4 and 5.25 showing P value < 0.025, 0.05 and 0.025 for the three parameters respectively.

7. Ulnar nerve- Motor

Recordings of this nerve in group A were 3.23, 43.9 and 5.3 while in group B were 3.49, 42.7 and 4.7 showing P value < 0.025, 0.05 and < 0.025 for the three parameters respectively.

Table 2: NCS in normal subject. (Control) group.

	Nerve	A (Female)				B (Male)				C (Mixed)			
		Lat	Vel	Amp	Fbs	Lat	Vel	Amp	Fbs	Lat	Vel	Amp	Fbs
1	Sura±l Nerve	3.0 ± 0.395	43.14 ±5.31	6.36 ± 2.5	92.0 ± 17.15	2.86 ± 0.5	44.17 ±5.49	6.7 ±2.3	90 ± 14	2.93 ±0.42	43.65 ±5.4	6.57 ±2.4	91.16 ± 15.5
						N	N	N	N				
2	Common Peroneal	3.856 ± 0.43	4.763 ±3.89	3.98± 1.73		3.34 ± 0.5	44.42 ±5.13	6.2 ± 2.0		3.59 ±0.46	46.02 ±4.2	5.11 ± 1.8	
						N	N	N					
3	Posterior Tibial	3.632 ± 0.62	45.64 ±5.65	4.9 ± 1.95		3.62 ± 0.6	42.6 + 4.9	4.5 ± 1.8		3.63 ± 0.6	44.12 ±5.2	4.73 ± 1.85	
						N	N	N					
4	Median Sensory	2.80 ± 0.50	47.11 ±5.43	8.6 ± 2.6		2.69 ± 0.3	48.3 ± 4.2	7.3 ± 1.92		2.74 ± 0.4	47.7 ± 4.7	8 ± 2.3	
						N	N	N					
5	Median Motor	3.16 ± 0.548	51.44 ±5.4	5.31 ± 2.0		3.21 ± 0.3	49.0 ± 4.6	6.0 ± 2.0		3.18 ± 0.4	50.2 ± 5	5.7 ± 2	
						N	N	N					
6	Ulnar Sensory	2.346 ± 0.422	48.04 ±6.5	8.6 ± 2.4		2.33 ± 0.3	47.8 ± 5.6	7.3 ± 1.8		2.34 ± 0.4	47.9 ± 6	7.9 ± 2.2	
						N	N	N					
7	Ulnar Motor	2.59 ± 0.58	52.59 ±5.9	6.4 ± 2.0		2.57 ± 0.5	48.8± 5.62	6.1 ± 1.5		2.58 ± 0.52	50.71 ±5.7	6.2 ± 1.8	
						N	N	N					

N = Not significant
Latency in millisecond.
Velocity in meter / second
Amplitude in millivolts.

Table 3: Effect of duration on Nerve conduction Study in IDDM patients.

Nerve	Group A (< 10y)			Group B (> 10y)		
	Latency	**Velocity**	**Amp**	**Latency**	**Velocity**	**Amp**
Sural	4.8 1.23	38.88 6.4	5.3 2.1	4.56 1.05	35.4 5.3	3.8 1.8
P value	0.01 , 0.05 , 0.05					
Common Peroneal	4.86 1.24	39.7 6.7	3.69 1.4	5.29 1.4	36.12 5	3.0 1.5
P value	0.05 , 0.05 , 0.025					
Posterior Tibial	4.64 1.02	38.9 6.5	3.58 1.3	5.25 1.38	36.41 4.7	3.2 1.2
P value	0.05 , 0.05 , N					
Median Sensory	3.82 0.99	40.1 5.7	4.91 1.4	4.02 1.03	39.2 8.6	4.42 1.4
P value	N , 0.025 , 0.025					
Median Motor	3.44 0.99	42.93 5.8	4.8 1.9	4.67 1.2	40.2 8.7	4.4 1.45
P value	0.025 , 0.05 , 0.025					
Ulnar Sensory	2.99 0.69	43.13 8.6	5.23 2.2	3.21 0.96	40.55 9.6	5.6 1.8
P value	0.01 , 0.05 , 0.025					
Ulnar Motor	2.98 1.26	45.7 7.4	5.27 2.3	3.7 0.06	43.9 8.5	5.0 2.4
P value	**0.05 , 0.05 , N**					

Latency in millisecond
Velocity in meter / second
Amplitude in millivolts.

Table 4: Effect of duration on Nerve conduction Study in NIDDM patients.

Nerve	Group A (< 10y)			Group B (> 10y)		
	Latency	**Velocity**	**Amp**	**Latency**	**Velocity**	**Amp**
Sural	4.7 1.2	38.75 4.47	4.02 1.02	5.1 1.4	35.32 5.6	3.68 0.8
P value	0.05 , 0.005 , 0.05					
Common Peroneal	4.9 2.2	42.2 7.6	3.5 0.96	5.26 .98	36.5 5.2	3.2 0.7
P value	0.05 , 0.001 , N					
Posterior Tibial	5.3 1.94	41.78 5.9	3.62 1.6	5.8 2.2	36.3 5.2	3.2 1.04
P value	0.025 , 0.05 , N					
Median Sensory	3.75 0.47	41.5 6.2	5.1 2.2	4.25 1.02	38.72 6.6	4.5 1.6
P value	0.05 , 0.005 , 0.5					
Median Motor	4.27 2.0	47.2 8.8	4.32 1.09	4.86 1.4	38.32 7.4	3.0 1.75
P value	0.05 , 0.005 , 0.05					
Ulnar Sensory	3.2 0.86	42.5 7.4	6.0 2.1	3.6 1.03	39.4 8.6	5.25 2.2
P value	0.025 , 0.05 , 0.25					
Ulnar Motor	3.23 0.094	43.9 8.7	5.3 2.3	3.49 1.1	42.7 9.6	4.7 2.04
P value	**0.025 , 0.5 , 0.25**					

Latency in millisecond
Velocity in meter / second
Amplitude in millivolts.

Discussion and conclusion

The present study shows no significant differnces between the males and females (control) for latency, velocity and amplitude recordings Thus the two groups (males and females) are regareded as one group to which the obtained results of the diabetic group (first visit) are compared.

Electrophysiological measurments in terms of increased latencies with reduction in sensory conductive velocity (SCV) and motor conductive velocity (MCV) along with reduction in amplitude was found more evident in diabetic patients with more than 10 years duration; (IDDM and NIDDM) showed significant difference in the above parameters when compared with the results obtained from diabetic patients of shorter duration (P range 0.01- 0.005). This finding is in consistance with [26-28], who found that duration of diabetes correlates positively with neuropathy in patients who had diabetes for 10 years.

People with diabetes are prone to compressive neuropathies which might account for changes in electrophysiological tests [29]. This report support the present findings of prolonged distal latencies in all the tested nerve including the sural nerve which can not be comprssed. The distal latency of the sural nerve affected in 88.9% of the tested patients, while the ulnar nerve is affected in (44.4% for sensory and 55.5% for motor) and the median nerve in (66.6% for sensory and 55.5% motor). These findings are highly consistent with the dying back neuropathy in which the distal nerves are more affected than proximal nerves. This phenomenon can be considered as a support for the hypothesis that diabetic neuropathy is at least partially a result of diabetic angiopathy.

This is also in agreement with [29] who stated that prolonged distal latencies may be a very early marker for those who will be continue to develop more sigificant nerve damage. The dying back neuropathy is known to be one type of axonal atrophy [30].

The study of amplitude of sensory and motor nerves were found to be affected since the first visit (88.8% for sensory and 74.07% for motor nerves). In this case the tested patients were highly hyperglycemic indicating metabolic derangement that may resulted in reduction in the

number of functioning axons within the nerve. This finding is consistent with (31-32) who found that the most significant relationship is occurred between the glycemic control and the amplitude of the median and sural nerves. Similar results were obtained in the present study, since the sural nerve at first visit of the patients showed the highest changes for the velocity (88.9%) and amplitude (77.8%).

The median sensory and ulnar motor velocity have changed in 96.29% of the tested patients, so they are good markers for neuropathy in the upper extremity.

The above obtained data for latency, velocity and amplitude are consistent with [33] in that symptomatic and asymptomatic neuropathy nerves have a mixture of abnormal fibers of segmental de and re-myelination and other fibers that undergo axonal degeneration.

According to the findings of the present work, the conclusion seems to be compatible with the hypothesis of mixed metabolic – vascular pathogenesis of diabetic neuropathy.

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